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THE OPSONIC INDEX IN ACUTE ARTICULAR RHEUMATISM.*

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A NUMBER of observers maintain that they have found a specific micro-organism in acute articular rheumatism. That acute articular rheumatism is a specific disease produced by a specific organism is claimed by Triboulet and Apert, Westphal, Wassermann and Malkoff, Fritz Meyer, Poynton and Paine, Beaton and Walker, Beattie, Lewis and Longcope, and others. This claim is based upon the fact that in a number of cases of this disease a micrococcus has been isolated which produces in rabbits and monkeys polyarthritis, endocarditis, and other manifestations of acute articular rheumatism. The observers admit that this organism, *M. rheumaticus*, cannot be differentiated morphologically or culturally from *Strept. pyogenes*. It is only by the production of the disease in animals that it shows its specificity. For example, Beattie¹ finds that inoculation into rabbits of *M. rheumaticus* produces a non-purulent arthritis in 60 per cent and endocarditis in 33 per cent while *Strept. pyogenes* causes a purulent arthritis in 18 per cent and endocarditis in only 2 per cent of the animals.

On the other side Cole² maintains that "arthritis and endocarditis may be produced by the intravenous inoculation of rabbits with streptococci from various sources, and the results obtained are quite similar to those described as resulting from the inoculation of the so-called *Micrococcus* or *Diplococcus rheumaticus*." He considers it unwarranted to establish a distinct variety of streptococci based on this property of causing endocarditis and arthritis.

Meakins³ has recently corroborated the findings of Cole, producing non-purulent arthritis by the intravenous inoculation of rabbits with various strains of streptococci.

Inasmuch as the specific opsonic index in certain acute infectious

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¹ *Brit. Med. Jour.*, 1906, 2, p. 1781.

³ *Canad. Jour. Med. and Surg.*, 1909, 25, p. 71.

² *Jour. Infect. Dis.*, 1904, 1, p. 714.

diseases follows a definite and typical course, it was thought that it might prove of interest to determine the course of the index to *M. rheumaticus* and streptococci in acute articular rheumatism.

Two strains of *M. rheumaticus* were employed. One was kindly furnished by Professor Beattie, the other came from the laboratory of Poynton and Paine.

An effort was made to isolate similar streptococci from the patients under observation in order to determine the opsonic index to the homologous organism. Blood cultures were made in three of the most severe cases in broth and milk and broth slightly acidified with lactic acid as suggested by Poynton and Paine.¹ The cultures all remained sterile.

Philipp,² Cole,³ Beattie,⁴ Marchildon,⁵ and others also failed to obtain positive results from blood cultures. Cultures of the joint exudate (by Dr. Irons) in one case of my series proved sterile. This also agrees with the results of the writers just mentioned. However, Loeb⁶ isolated a streptococcus from the blood in seven out of 45 cases of acute articular rheumatism, from a joint of one case, and from the blood of one patient with chorea, the nine strains corresponding culturally and morphologically to those described by Triboulet and Apert, Wassermann, Poynton and Paine, but he failed to produce with them arthritis in rabbits. Loeb believes it unlikely that the strains isolated by him are identical with those cultivated by Wassermann, Poynton and Paine, and others.

Meyer⁷ and Menzer⁸ isolated from the throats of patients with acute rheumatism streptococci which in rabbits produced multiple arthritis and often vegetative endocarditis. Hence throat cultures were made in eight of my cases, streptococci being isolated by means of blood-agar plates, human, rabbit, and sheep blood giving similar results. In one case the number of green colonies and hemolysing colonies of streptococci were about equal in number. The plates from two other cases showed three or four hemolysing colonies, the rest, several thousand, being green. In the remaining five cases the plates showed green colonies only. These results differ somewhat from those of Dr. D. J. Davis⁹ who found an increase in the number of hemolysing streptococci in the throats of all of the cases of acute articular rheumatism he examined. Occasionally there was seen slight hemolysis around the greenish colonies, the corpuscles remaining intact immediately around the colony, as is frequently seen in 48-hour pneumococcus colonies. A greenish-brown discoloration in blood-agar cultures of *M. rheumaticus* has been described by Shaw¹⁰ and by Walker.¹¹ The two strains of this organism studied by me (Beattie, Poynton and Paine) also produced similar green colonies in plates with human, rabbit, and sheep blood. The Poynton and Paine organism sometimes showed slight hemolysis around the green colonies in 48-hour plates.

Greenish color has been shown to be produced by pneumococci (Schottmüller,¹² Rosenow¹³) and by various strains of streptococci (Schottmüller), called by the latter *Strept. viridans*. The pneumococcus is differentiated from *Strept. viridans* by its

¹ *Practitioner*, 1901, 66, p. 22.

² *Deut. Arch. f. klin. Med.*, 1903, 76, p. 150.

³ *Jour. Infect. Dis.*, 1904, 1, p. 714.

⁴ *Brit. Med. Jour.*, 1906, 2, p. 1781.

⁵ *St. Louis Med. Rev.*, 1908, 57, p. 185.

⁶ *Arch. Int. Med.*, 1908, 2, p. 266.

⁷ *Deut. med. Wchnschr.*, 1901, 27, p. 81.

⁸ *Ibid.*, 1901, 27, p. 97.

⁹ Personal communication.

¹⁰ *Jour. Path. and Bact.*, 1904, 9, p. 158.

¹¹ *Brit. Med. Jour.*, 1907, 1, p. 1233.

¹² *Munch. med. Wchnschr.*, 1903, 50, p. 909.

¹³ *Jour. Infect. Dis.*, 1904, 1, p. 308.

power to ferment inulin. Now, the two cultures of *M. rheumaticus* and the eight strains of streptococci producing green colonies on blood-agar plates, isolated from the throat in rheumatism, did not ferment inulin.

Ruediger¹ found green-producing colonies in all normal and nearly all diseased throats. He considers that they belong to a large group of organisms between typical pneumococci and streptococci. Some seem to correspond to Schottmüller's *Strept. viridans*, but others are closely related to the pneumococcus. He believes that all of these organisms including the *Strept. viridans* are closely related to one another and can be sharply differentiated from *Strept. pyogenes* by the following characteristics:

1. The cocci in smears made from milk cultures stain rather poorly and unevenly with Löffler's methylene blue, are usually somewhat elongated or lance-shaped, and often are found in pairs. *Strept. pyogenes* takes the methylene-blue stain readily, the cocci are never distinctly grouped in pairs in the chains and are not elongated, but frequently appear to be disc-shaped.

2. On ascites-agar slants a large proportion of these atypical organisms form lance-shaped or oval cocci which are grouped chiefly in pairs, but also in short chains.

3. When pure cultures are grown on serum-glucose-agar slants (three parts of glucose agar to one part ascites fluid or serum) no change is produced in the medium while *Strept. pyogenes* turns it white and opaque in from 36 to 48 hours. According to Libman the pneumococcus produces no such change.

Smears from milk cultures of the strains of green-producing cocci isolated by me from the throat in rheumatism showed faintly staining chains in six of the eight cultures. None showed any elongation of the cocci. The other two strains, the strains of *M. rheumaticus* and *Strept. viridans* from a normal throat, stained perfectly distinctly, as did the strains of *Strept. pyogenes* (one being obtained from the throat in a case of rheumatism). On ascites agar three of the six strains of green-producing colonies from the throat in rheumatism showed oval or lance-shaped cocci. The three other strains and *M. rheumaticus*, *Strept. viridans* (normal throat), and *Strept. pyogenes* were indistinguishable morphologically.

On ascites-glucose agar the strains of *Strept. pyogenes*, *M. rheumaticus*, and three strains of the green-producing cocci produced much opacity. The other green-producing strains and *Strept. viridans* (normal throat) produced only slight opacity. Rosenow found that strains of pneumococci after isolation acquire the ability to cause opacity in serum-glucose agar. This proved true of two of four of the pneumococcus cultures that I tested. It would seem, then, that this means of differentiation cannot be considered conclusively accurate. Although these strains of streptococci show differences morphologically, just as they do culturally in their growth in milk, these are not sufficient to differentiate them. None of the green-producing organisms correspond exactly to the classification of Ruediger. The two strains causing slight opacity in ascites-glucose agar, staining faintly in smears from milk and appearing as lance-shaped cocci on ascites-agar slants, come the nearest to corresponding to the organisms described by Ruediger.

Gordon and Houston² and later Andrews and Horter³ have classified streptococci according to their different chemical reactions on various media. According to Andrews and Horter a strain of *M. rheumaticus* from Paine is classified as *Streptococcus salivarius*; one from Beattie as *Streptococcus faecalis*. The authors find that

¹ *Jour. Infect. Dis.*, 1906, 3, p. 755.

³ *Ibid.*, 1906, 2, pp. 708, 775, 852.

² *Lancet*, 1905, 2, p. 1400.

"*Streptococcus salivarius* passes by insensible gradations into *Streptococcus faecalis*." They believe that Schottmüller's hemolysing *Strept. pyogenes* corresponds to theirs, and that his *Strept. viridans* probably corresponds in general to their *Strept. salivarius* and *Strept. faecalis*. According to this classification the strains of *Strept. viridans* isolated by me from throats of rheumatism patients would belong to the same class as the strains of *M. rheumaticus* of Paine and Beattie.

Meakins¹ has examined eight strains of *M. rheumaticus* according to this method and finds that it "is not a distinct organism separate from other streptococci."

Beattie² states that *M. rheumaticus* can be differentiated from other streptococci by the production of acid and the precipitation of bile salts in McConkey's lactose broth. Meakins tested 25 strains of streptococci from different sources as to their reaction with this medium and found that the reaction was not peculiar to streptococci isolated from rheumatic patients.

Walker³ finds that the streptococcus isolated from cases of rheumatism produces considerably more formic acid than is produced by streptococci from other sources. This might be a means of differentiation, but, as Cole points out, this test which is a quantitative one will probably not be of value in the study of an organism as variable in its characteristics as the streptococcus.

The only characteristic which seems to differentiate the green-producing streptococci isolated by me and the strains of *M. rheumaticus* (Beattie, Poynton and Paine) from *Strept. pyogenes* is the production of green colonies in blood-agar plates. On account of this property and their not fermenting inulin they may be considered as belonging to the streptococcus viridans group and in the rest of this paper the organisms isolated by me will be called *Strept. viridans* to distinguish them from the *M. rheumaticus* (Beattie, Poynton and Paine).

OPSONINS IN ACUTE ARTICULAR RHEUMATISM.

The opsonic index to various organisms has been estimated in the usual way in 18 cases of typical acute articular rheumatism. Ten were examined usually every other day during the course of the disease. In eight the index was observed only twice, the patients coming under observation at the end of the disease. The patients were all hospital cases. With one exception they had been ill from one to three weeks before entrance. However, in three cases (Charts 3, 4, 5) new joints became involved after admission, so that the indices could be estimated under these circumstances. The indices were taken to *M. rheumaticus* (Beattie, Poynton and Paine), *Strept. pyogenes*, *Strept. viridans*, and the pneumococcus. The opsonic index to the *Staph. aureus* and *Strept. viridans* (normal throat) were also estimated in the first six cases.

¹ *Canad. Jour. Med. and Surg.*, 1909, 25, p. 71.

² *Brit. Med. Jour.*, 1906, 2, p. 1781.

³ *Brit. Med. Jour.*, 1907, 1, p. 1233.

In the charts *M. rheumaticus* refers either to the Beattie or to the Poynton and Paine organism. The two organisms were

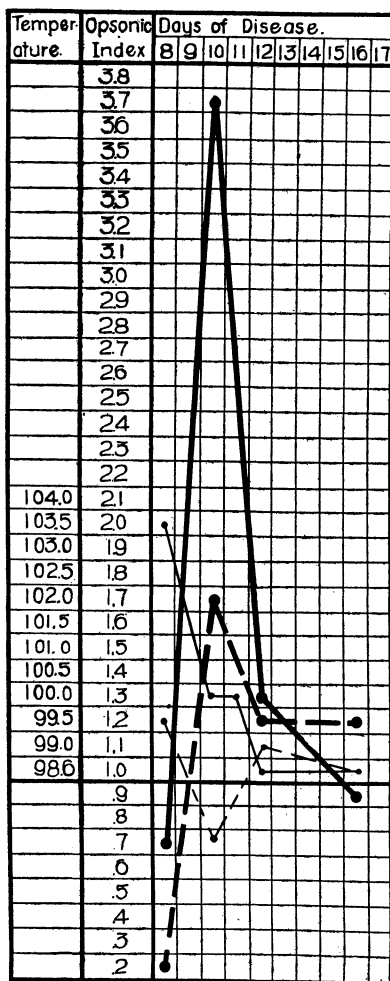


CHART 1.—ACUTE ARTICULAR RHEUMATISM (WOMAN, AGE 50).

Heavy solid line = Opsonic index to *M. rheumaticus*.

Heavy broken line = " " " *Strept. pyogenes*.

Fine broken line = " " " *Pneumococcus*.

Fine solid line = Temperature.

frequently both used and gave similar results. The strains of *Strept. pyogenes* were isolated from scarlet fever and erysipelas patients. Often two or more strains were employed but the opsonic index was the

same to the various strains, as I found to be the case also in my work on the index in scarlet fever and erysipelas. The homologous *Strept.*

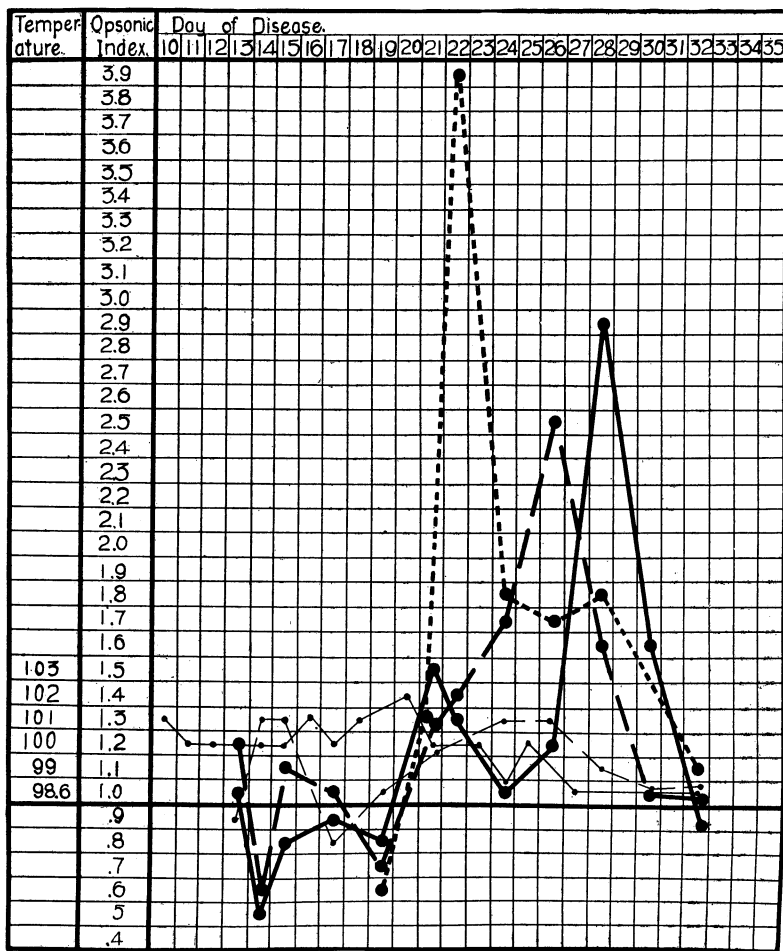


CHART 2.—ACUTE ARTICULAR RHEUMATISM AND ENDOCARDITIS (WOMAN, AGE 20).

Heavy solid line = Opsonic index to *M. rheumaticus*.

Heavy broken line = " " " *Strept. pyogenes*.

Fine broken line = " " " *Pneumococcus*.

Heavy dotted line = " " " Homologous *Strept. viridans*.

Fine solid line = Temperature.

viridans was employed in five cases, in one case (Chart 6) both the homologous *Strept. viridans* and the homologous *Strept. pyogenes*.

The opsonic index to the strains of *Strept. viridans* isolated from

the throat in rheumatism was frequently estimated in other patients and the index closely corresponded to the homologous index. It was often difficult to obtain a sufficiently smooth suspension with the freshly isolated bacteria before the recovery of the patient. On this account and because the bacteria are frequently not phagocytatable until grown on artificial culture media for several generations, the

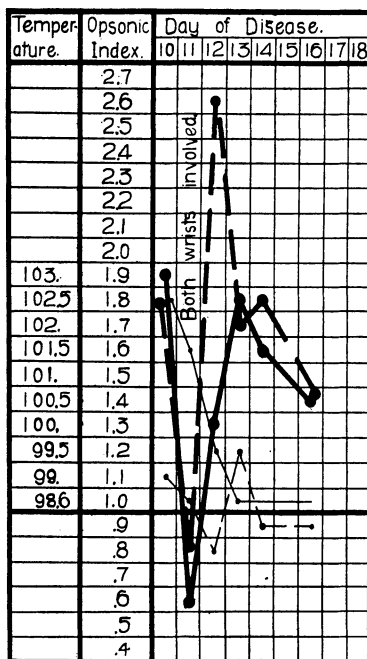


CHART 3.—ACUTE ARTICULAR RHEUMATISM (MAN, AGE 40).

Heavy solid line = Opsonic index to *M. rheumaticus*.
 Heavy broken line = " " " *Strept. pyogenes*.
 Fine broken line = " " " *Pneumococcus*.
 Fine solid line = Temperature.

homologous organisms could not always be employed. It was found that the opsonic index followed the same course in the case of *M. rheumaticus* (Beattie, Poynton and Paine), *Strept. pyogenes*, and *Strept. viridans* (from patients with rheumatism) while it remained within the normal limits with respect to *Staph. aureus*, and, except in one case, pneumococcus and *Strept. viridans* from normal throat.

In the five cases examined before improvement commenced the opsonic index for *M. rheumaticus*, *Strept. pyogenes*, and

Strept. viridans (rheumatism) was found below normal—0.2–0.5 (Charts 1 and 2). With the involvement of new joints the index to these strains would fall to 0.5–0.7 (Charts 3, 4, 5). In all of the 18 cases just before the fall in temperature and improvement in the joints the opsonic index for the three strains of streptococci rose above normal, the average being 2.5. The charts show that the

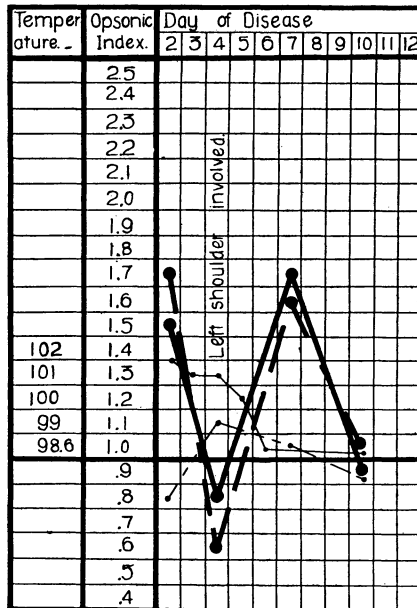


CHART 4.—ACUTE ARTICULAR RHEUMATISM AND ENDOCARDITIS, FOURTH ATTACK (MAN, AGE 35).

Heavy solid line = Opsonic index to *M. rheumaticus*.
 Heavy broken line = " " " *Strept. pyogenes*.
 Fine broken line = " " " *Pneumococcus*.
 Fine solid line = Temperature.

indices for *M. rheumaticus*, *Strept. pyogenes*, and *Strept. viridans* (rheumatism) follow the same course and are often identical. In Chart 2 the difference perhaps appears greater than it really is, the indices all being above normal during about the same period. In one case (Chart 5) there was an increase in opsonin for pneumococcus and *Strept. viridans* (normal) but not coincidentally with the rise in the opsonic index for *M. rheumaticus* and *Strept. pyogenes*. No evident explanation could be found for this rise, the patient being carefully examined for pneumococcal complications. These results

agree with those obtained by me¹ in studying the opsonic index in scarlet fever. Here, too, the opsonic index for *Strept. viridans* (normal) followed the course of the pneumococcus and not that of *Strept. pyogenes*.

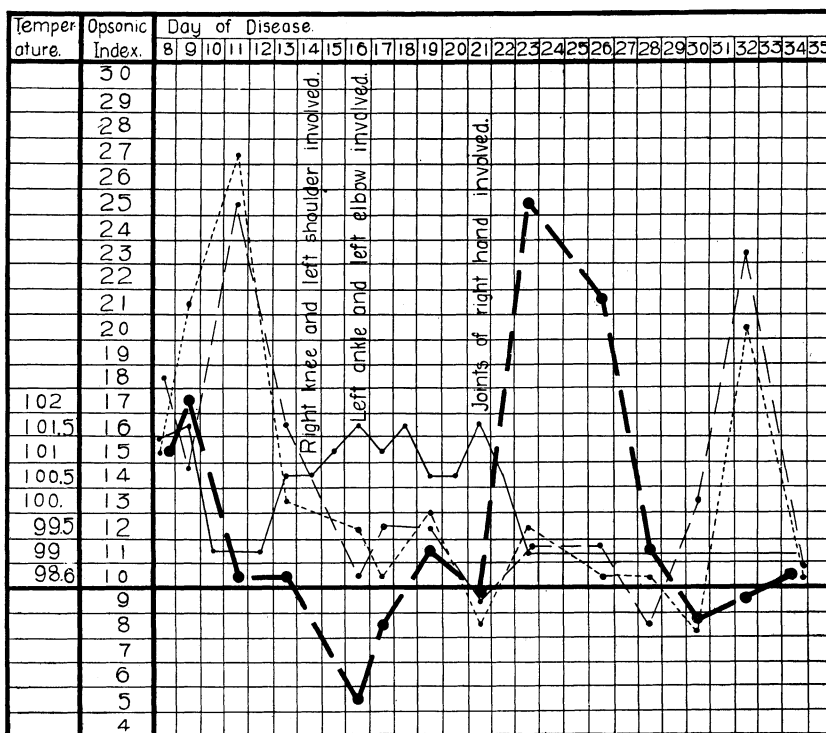


CHART 5.—ACUTE ARTICULAR RHEUMATISM (MAN, AGE 30).

Heavy broken line = Opsonic index to *Strept. pyogenes*.

Fine broken line = " " " Pneumococcus.

Fine dotted line = " " " *Strept. viridans* (Normal throat).

Fine solid line = Temperature.

In one case (Chart 6) from the throat of which both *Strept. pyogenes* and *Strept. viridans* were isolated, the indices for both these organisms correspond to the indices for *Strept. pyogenes* (scarlet fever) and *M. rheumaticus*. The study of the opsonic index in acute articular rheumatism to streptococci producing green colonies on blood-agar plates would suggest that this group of organisms isolated from the throat in rheumatism is closely related to the *Strept. pyogenes*.

¹ *Jour. Infect. Dis.*, 1907, 4, p. 304.

The opsonic indices in these experiments have been estimated in the usual way. From time to time the results have been controlled both by heating the normal and immune (patient's) serum to 46° for 15 minutes, and by diluting the serum to the point of opsonic extinction, or more correctly the point exceeding spontaneous

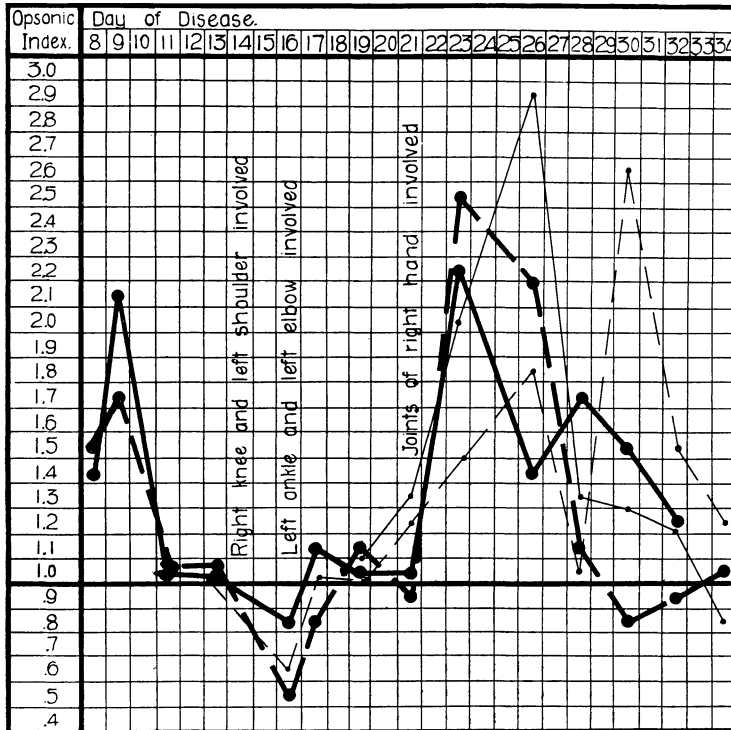


CHART. 6.—ACUTE ARTICULAR RHEUMATISM (MAN, AGE 30). (Same patient as in Chart 5.)

Heavy solid line = Opsonic index to *M. rheumaticus*.
 Heavy broken line = " " " *Strept. pyogenes*.
 Solid fine line = " " " Homologous *Strept. viridans*.
 Broken fine line = " " " " *Strept. pyogenes*.

phagocytosis. For example, the opsonic indices obtained in the usual way for *M. rheumaticus* and *Strept. pyogenes* were 1.8 and 1.7; after heating the normal and immune serum to 46° for 15 minutes the indices were 2.7 and 2.8 respectively. With an index for *M. rheumaticus* of 1.4 with undiluted serum, on diluting the serum; the point of opsonic extinction for the normal was 1:24 while that of the immune serum was 1:94. One patient had an index of

0.6 for *Strept. pyogenes* determined in the usual way. On dilution of the serum, the point of opsonic extinction for the normal serum was 1:12 while that of the patient's serum was 1:6. Two days later with an index of 3.4 for the same organism the point of opsonic extinction for the normal serum was found to be 1:24 while that of the patient's was 1:94.

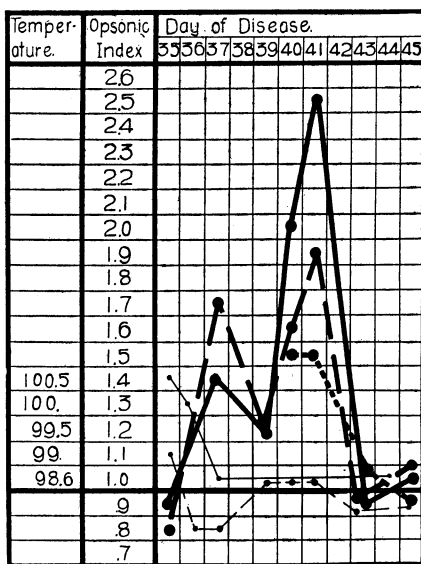


CHART 7.—ACUTE ARTICULAR RHEUMATISM (WOMAN, AGE 40)

Heavy solid line = Opsonic index to *M. rheumaticus*.
 Heavy broken line = " " " *Strept. pyogenes*.
 Heavy dotted line = " " " Homologous *Strept. viridans*.
 Fine broken line = " " " Pneumococcus.
 Fine solid line = Temperature.

These results correspond in general with those obtained in diphtheria and erysipelas in which I also found that the course of the indices determined by heating and by diluting the serum corresponded closely with that obtained by the Wright method.¹ Klien² and Meakins³ found that when, by the usual method of determining the opsonic index, they obtained normal indices, diluting the serum to the point of opsonic extinction brought out great differences between the

¹ *Jour. Infect. Dis.*, 1908, 5, pp. 14 and 238.

³ *Jour. Exp. Med.*, 1900, 2, p. 100.

² *Johns Hopkins Hosp. Bull.*, 1907, 18, p. 245.

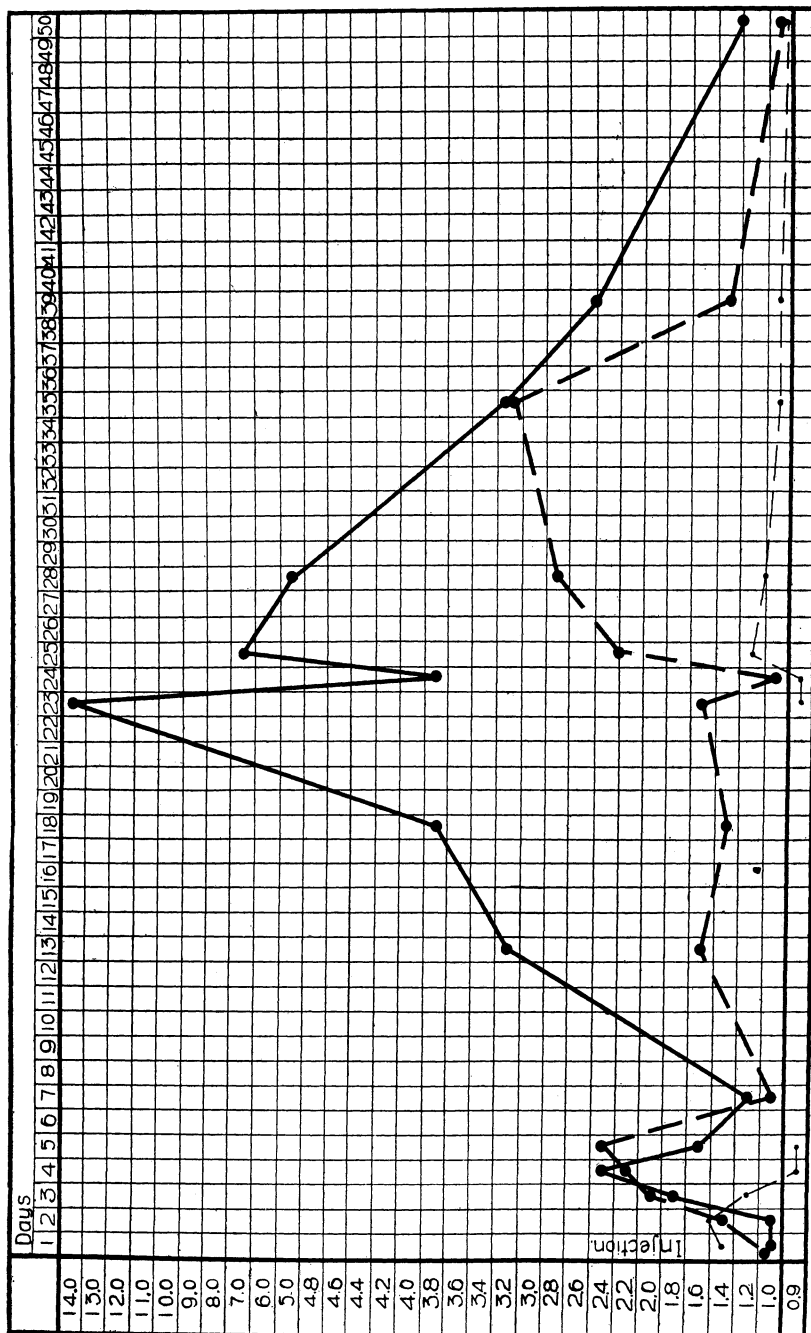


CHART 8.—OPSONIC CURVES OF RABBIT INJECTED INTRAVENOUSLY WITH 24-HOUR GROWTH OF FOUR BLOOD-AGAR SLANTS OF *M. rheumaticus* (Beattie).
 Heavy solid line = Opsonic index to *M. rheumaticus*.
 Heavy broken line = " " " *Strept. pyogenes*.
 Fine solid line = Opsonic index to *M. rheumaticus* (Beattie).
 Fine broken line = Opsonic index to *Pneumococcus*.

normal and immune sera. It is possible the thickness of their suspension was such that accurate counts with the undiluted serum were not possible.

A rabbit (Chart 8) was injected intravenously with a 24-hour growth of four blood-agar slants of *M. rheumaticus* (Beattie). The animal was slightly sick the day after the injection, but no arthritis developed. The opsonic index was taken at first for *M. rheumaticus*, *Strept. pyogenes*, pneumococcus, staphylococcus, *Strept. viridans* (normal, and from rheumatism three strains); later only for *M. rheumaticus*, *Strept. pyogenes*, and pneumococcus. The index for the pneumococcus, staphylococcus, and *Strept. viridans* (normal) remained within the normal variations. The indices for the other streptococci all closely corresponded in their course. The differences in the height of the indices for *M. rheumaticus* and *Strept. pyogenes* after the 12th day is owing probably to the virulency of *M. rheumaticus* (Beattie). I have found that in immunized animals great differences in the height of the index, but not in the course, may occur, if a virulent and non-virulent organism of the same strain are employed, the differences being due to the small opsonic effect of the normal serum on the virulent strain. From Chart 8 it is seen that the serum of the rabbit immunized with *M. rheumaticus* shows an increase in opsonin for both *M. rheumaticus* and *Strept. pyogenes*.

Agglutination experiments were made with the serum of the immunized rabbit and that of 12 rheumatic patients. The experiments were repeated three or four times during the course of the disease. In each case both *Strept. pyogenes* and *M. rheumaticus* and in one case the *Strept. viridans* (homologous) were employed. All of the tests were macroscopic. At first calcium broth cultures were used according to the method of Hiss,¹ the tubes being incubated for three hours and then placed in the ice chest for 18 hours. Later 0.2 per cent dextrose broth cultures was employed after centrifugalization for a few minutes to remove clumps. The tubes were incubated for three hours and then left at room temperature for 18 hours.

The agglutination experiments were often unsatisfactory and had

¹ *Jour. Exper. Med.*, 1905, 7, p. 560.

to be repeated on account of the spontaneous agglutination of the organisms in the broth cultures. No more agglutinin was found in the immune rabbit serum than in normal. Of the 12 patients examined, agglutinins to both *Strept. pyogenes* and *M. rheumaticus* were demonstrated in seven. It is probable that if the serum of the other five patients had been examined oftener, agglutinins would have been found, as they were present as a rule on one day only in the positive cases. During the negative phase of the streptococco-opsonic indices the agglutinins were normal or below, normal serum agglutinating both strains of streptococci at a dilution of about 1:10. With the rise in the opsonic indices, the agglutinative power increased in four cases, the highest dilution at which agglutination occurred being 1:100. In one case the increase in agglutinins occurred the day before and in two cases the day following the rise in the opsonic indices. The homologous *Strept. viridans* was also tested in one case. It was agglutinated at a dilution of 1:100 at the height of the opsonic indices and continued to be so agglutinated for seven days.

Meyer¹ found that streptococci are agglutinated by specific immune serum and he differentiates, by means of agglutination, the streptococci of anginal, scarlatinal, rheumatic infections from those of simple pyogenic infections. He also found gradual differences in the agglutination of the anginal streptococci which were interpreted as speaking against their identity. Aronson,² however, does not find that a differentiation can be made between groups of streptococci by agglutination. Wlassjewski³ found the serum from a case of rheumatism had no effect upon any of the streptococcus cultures employed (scarlet fever, puerperal fever, erysipelas, phlegmon, and dysentery). Weaver⁴ has found that the agglutination reaction between streptococci cultivated from cases of scarlatina and the serum from cases of scarlet fever is in no way specific, these streptococci being agglutinated at about the same dilution by sera from cases of lobar pneumonia, erysipelas, and typhoid fever, and to almost the same extent by puerperal-fever serum.

¹ *Deut. med. Wchnschr.*, 1902, 28, p. 751.

² *Ibid.*, 1903, 29, p. 439.

³ *Centralbl. f. Bact.*, Abt. I, 1903, 33, p. 464.

⁴ *Jour. Infect. Dis.*, 1904, 1, p. 91.

CONCLUSIONS.

In acute articular rheumatism the opsonic index for *M. rheumaticus* (Beattie, Poynton and Paine) and *Strept. pyogenes* followed the same course. With involvement of new joints and rise in temperature the indices for both these organisms fell below normal. With improvement in the joints and symptoms the indices rose above normal.

The opsonic index for *Strept. viridans* isolated from the throat in acute rheumatism followed the same course as the index for *M. rheumaticus* and *Strept. pyogenes*.

The indices for *Staph. aureus*, pneumococcus, and a strain of *Strept. viridans* from a normal throat remained within the normal limits.

The opsonic index consequently does not help to differentiate *M. rheumaticus* from *Strept. pyogenes*.

A rabbit immunized with *M. rheumaticus* gave an increase in opsonin for *M. rheumaticus*, *Strept. pyogenes*, and *Strept. viridans* (rheumatism), but none for *Staph. aureus*, pneumococcus, and *Strept. viridans* (normal).

Agglutinins to both *Strept. pyogenes* and *M. rheumaticus* were demonstrated in the serum of seven of 12 rheumatism patients, the course of the agglutinins corresponding in general to that of the opsonic indices.

From the results of this study the conclusion seems warranted that streptococci play an essential part in acute articular rheumatism.